

SUMMARY

[0011] The disclosure provides flavin-dependent halogenases (FDHs) that are amino acid variants of fungal MalA or MalA' halogenases useful in specifically halogenating complex compounds such as indole alkaloids. These enzymes are useful in producing a variety of halogenated compounds in the fungal malbrancheamide pathway that have physiological effects in mammals such as inhibiting calmodulin and, thereby, modulating calcium ion signaling pathways involved in a diverse set of physiological pathways and, hence, a variety of diseases or disorders. Exemplary methods comprising administration of effective amounts of the compounds disclosed herein include inhibiting the contraction of smooth muscle in a mammal.

[0012] In one aspect, the disclosure provides a flavin-dependent halogenase (FDH) variant comprising one or two amino acid substitutions compared to the amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:4, wherein the FDH variant is capable of catalyzing the halogenation of a complex organic compound. In some embodiments, the complex organic compound is an aromatic heterocyclic organic compound. In some embodiments, the aromatic heterocyclic organic compound comprises a bicyclo[2.2.2]diazaoctane ring. In some embodiments, the aromatic heterocyclic organic compound comprises indole. In some embodiments, the compound is an indole alkaloid. In some embodiments, the indole alkaloid is a prenylated indole alkaloid. In some embodiments, the FDH variant is derived from a fungal FDH. In some embodiments, the FDH variant is derived from a bacterial FDH. In some embodiments, the FDH variant is not derived from a fungal FDH or a bacterial FDH.

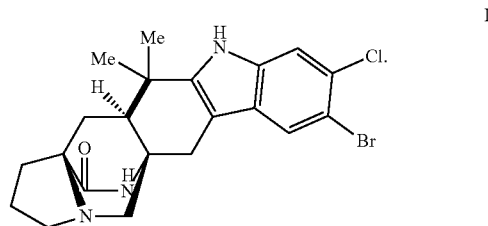
[0013] In some embodiments, the FDH variant is a MalA halogenase variant. In some embodiments, the FDH variant comprises an amino acid substitution variant of the sequence set forth in SEQ ID NO:2, wherein the substitution is S129Z, H253Z, S129Z/H253Z, D109Z, F489Z, S409Z, W265Z, W263Z, S82Z, S129Z/G131Z, G131Z, S129Z/I493Z, I493Z, S129Z/P85Z, or P85Z, wherein Z is no amino acid or any conventional amino acid except the wild-type amino acid at the indicated position. In the context of the disclosure, a substitution mutation or variation at the amino acid level is expressly defined as including the substitution of any naturally occurring conventional amino acid for a non-identical amino acid, or the substitution of no amino acid for a given amino acid, i.e., a single amino acid deletion. In some embodiments, the FDH variant comprises an amino acid substitution variant of the sequence set forth in SEQ ID NO:2, wherein the substitution is S129A, H253F, S129A/H253F, H253A, D109A, F489H, S409A, W265A, W263A, S82A, S129A/G131S, G131S, S129A/I493S, I493S, S129A/P85S, or P85S. In some embodiments, the MalA halogenase comprises the sequence set forth in SEQ ID NO:6, 8, 12, or 16. In some embodiments, the FDH variant is a MalA' halogenase variant. In some embodiments, the FDH variant comprises an amino acid substitution variant of the sequence set forth in SEQ ID NO:4, wherein the substitution is E494Z or H253Z, wherein Z is no amino acid or any conventional amino acid except the wild-type amino acid at the indicated position. In some embodiments, the FDH variant comprises an amino acid substitution variant of the sequence set forth in SEQ ID NO:4, wherein the substitution is E494D, H253F, S129A, S129A/I493S, I493S, S129A/P85S, P85S, S129A/G131S, or G131S. In some

embodiments, the MalA' halogenase comprises the sequence set forth in SEQ ID NO:10, 14, or 18.

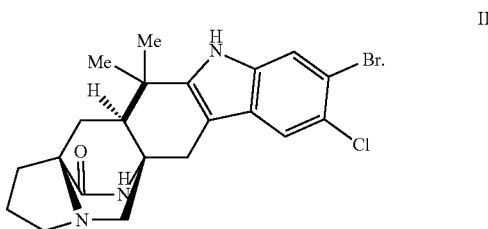
[0014] Another aspect of the disclosure is drawn to a polynucleotide encoding a FDH variant disclosed herein. In some embodiments, the encoded FDH variant is a MalA halogenase variant or a MalA' halogenase variant. In some embodiments, the encoded FDH variant comprises a sequence set forth in SEQ ID NO:6, 8, 10, 12, 14, 16, or 18.

[0015] Yet another aspect of the disclosure is directed to a vector comprising the polynucleotide disclosed herein. A related aspect of the disclosure is a host cell comprising the polynucleotide disclosed herein or the vector disclosed herein.

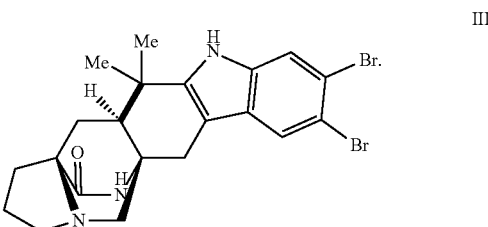
[0016] Another aspect of the disclosure is a malbrancheamide D compound comprising formula I:



[0017] A related aspect of the disclosure is an isomalbrancheamide D compound comprising formula II:



[0018] A related aspect of the disclosure is a malbrancheamide E compound comprising formula III:



[0019] Still another aspect of the disclosure is a method of halogenating a complex organic compound comprising contacting a complex organic compound with a FDH halogenase variant disclosed herein and a halogen under conditions suitable for enzyme-catalyzed halogenation of the complex organic compound. In some embodiments, the complex organic compound is an aromatic heterocyclic organic compound. In some embodiments, the aromatic heterocyclic organic compound comprises a bicyclo[2.2.2]diazaoctane